rearrangement due to cross-linking lipids in the headgroup position. Building on this, our current efforts investigate peptide perturbations in lipid bilayers. We cross-link transmembrane peptides on the surface of lipid vesicles to examine the interactions between the helices, mimicking B cell receptor clustering. Expanding on surface-based perturbations, we utilize peptide forming fibrils that associate with anionic lipids, indicating an electrostatic association. These fibril-liposome systems are a useful model to study plaque-based diseases such as Alzheimer's, Parkinson's and type II diabetes. We analyze these associations using microscopy, FRAP, FRET and CD spectroscopy.

1115-Plat
Lipid Bilayer Membrane Shape Remodeling by Lipid-Modified Ras Proteins
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Ras proteins are small GTP-hydrohydrolyzing enzymes that operate as molecular switches in signal transduction pathways and are present in a mutant, activated state in many human tumors. It has been shown that the lipid-modified C-terminus drive lateral segregation of Ras proteins into membrane sub-structure on the plasma membrane. Such transient and dynamic molecular assembly, so-call nanocluster, is emerging as a crucial mechanism by which cells achieve high-fidelity signal transmission. However, little is known about the underlying force driving the formation of nanoclusters on the plasma membrane and their influence on the membrane structure.
To investigate the interaction between nanocluster and the membrane, we carried out extensive semi-atomistic molecular dynamics simulations of the C-terminal membrane-targeting motif of H-ras (tH) in a phase separated lipid bilayer composed of 2000 DPPC, DliPC and cholesterol molecules. We found out that at ambient temperature approximately 30–40% of tH molecules assemble into clusters of 4-9 proteins, and preferentially localize at the interface between the liquid-order and liquid-disorder phases of the membrane. In the nanoclusters, tH backbones preferably exhibits an extended conformation and align linearly at the phase interface. With such molecular-level organization, tH molecule act as a linactant at the interface and reduce line tension of the anchor-containing monolayer and induces shape transition of the overall membrane due to the tendency of the system to minimize the free energy. The resultant geometry of the membrane is determined by the different elastic bending modulus of different phases as well as the phase boundary line tension, which is strongly affected by the localization of the nanoclusters. Our findings were explained based on the domain-induced budding model and shed light on the interaction between membrane-bounded proteins and membrane domains, such as lipid rafts.

1116-Plat
Native Ligands Alter Integrin Sequestering but not Oligomerization in Model Membranes
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Distinct lipid environments are increasingly recognized as an important factor influencing membrane protein properties. However, the small size and transient nature of lipid heterogeneities typically found in plasma membranes significantly complicate a thorough analysis of this intriguing relationship at the cellular level. To address this challenging topic, we present a powerful model membrane platform based on a polymer-supported bilayer of well-defined lipid compositions, in which the sequestering and oligomerization state of reconstituted membrane proteins can be determined in the absence of artificial cross-linking agents using confocal fluorescence spectroscopy paired with a photon counting histogram (PCH) method, as well as epifluorescence microscopy. Specifically, integrins ($\alpha v\beta 3$ and $\alpha 5\beta 1$) were reconstituted into the polymer-tethered lipid bilayer using a modified Rigaud technique and their sequestering and oligomerization behavior was analyzed in raft-mimicking lipid mixtures before and after addition of native ligands (vitronectin and fibronectin). Our data show that ligand binding notably enhances the affinity of $\alpha v\beta 3$ and $\alpha 5\beta 1$ for cholesterol-enriched lipid environments without altering the integrin oligomerization state. These findings support a mechanism of ligand-induced conformational changes of membrane proteins that impacts protein-lipid interactions. Finally, we present results that illustrate how changes in lipid composition affect ligand-mediated integrin sequestering/oligomerization in the model membrane environment.

1117-Plat
Translational and Rotational Diffusion, and Conformational Dynamics of fd Virus on Freestanding Cationic Lipid Membranes
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Understanding of the mechanisms of interaction of macromolecules and colloidal particles with lipid membranes is far from complete, and the questions related to the effects of membrane local curvature and bending elasticity of the interacting partners are still largely unsolved. Recently, using single-molecule fluorescence video microscopy we discovered unexpected effects in interaction of single DNA molecules with freestanding cationic lipid membranes [1]. In the present contribution, using the same experimental approach, we investigate how much more rigid negatively charged particles - fd viruses [2] - behave upon electrostatic binding to freestanding cationic lipid membranes. We study the translational and rotational diffusion of fd viruses with the lengths in the range of $(1\text{-}11)\times 0.88$ μm bound to freestanding membranes and observe the crossover from the 2D membrane-controlled to 3D bulk fluid-controlled diffusion dynamics. For virus particles with lengths >4.4 μm we additionally study the effects of the membrane on their conformations and conformational dynamics.
[1] C. Herold, P. Schwille, and E. P. Petrov, Phys. Rev. Lett. 104 (2010) 148102.
[2] S. Fraden and Z. Dogic, in: Soft Matter, Vol. 2: Complex Colloidal Suspensions (eds. G. Gompper and M. Schick) Wiley-VCH, Weinheim, 2006, pp. 1–86.

1118-Plat
Lipid and Detergent Effects on the Kinetic Stability of the GPCR, Rhodopsin
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The photoreceptor, rhodopsin is a kinetically stable G-Protein Coupled Receptor that is located in rod outer segment disk membranes. Differential scanning calorimetry (DSC) studies have shown that rhodopsin exhibits an irreversible scan rate dependent endothermic transition (Tm) at approximately 72 oC. The activation energy for thermal denaturation (Eact) calculated from the scan rate dependence of the Tm is sensitive to the integrity of the lipid bilayer. It is also sensitive to proteolytic cleavage of the extramembraneous loops of rhodopsin. Here we investigate the influence of palmitoylation, membrane cholesterol and digitonin solubilization on rhodopsin kinetic stability. DSC experiments were performed using a MicroCal VP-DSC microcalorimeter. Samples were scanned at 15, 30, 60 and 90o/hr. Because the protein transitions are irreversible, a second scan was used to determine the baseline. Rhodopsin palmitoylation at cys 322 and cys 323 anchors the C-terminus to the membrane. The Eact for rhodopsin in disk membranes treated with hydroxylamine to remove these palmitate groups was approximately 35 Kcal/mole less than that of native rhodopsin. The Eact for rhodopsin was also determined at different membrane cholesterol levels. These data indicated a decrease in stability both above and below native membrane cholesterol levels. Finally, unlike solubilization in other detergents, solubilization in digitonin had little effect on rhodopsin kinetic stability.

1119-Plat
Ionization Properties of Phosphatidylinositol 4,5-Bisphosphate in Complex Ternary Lipid Systems
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Phosphatidylinositol 4,5-bisphosphate [$PI(4,5)P_2$] is an important signaling lipid that provides spatiotemporal control for a broad range of signaling events. $PI(4,5)P_2$ is a highly charged lipid, and electrostatics play a crucial role in protein/$PI(4,5)P_2$ interactions. The exact charge on $PI(4,5)P_2$ can be strongly affected by cellular conditions. We have used solid-state MAS ^{31}P-NMR to examine the ionization behavior of $PI(4,5)P_2$ in multilamellar vesicles containing mixtures of other lipids, for pH values from 4 to 10. Previous research has shown a complex ionization pattern for $PI(4,5)P_2$ in binary lipid mixtures of PC and $PI(4,5)P_2$ (Kooijman et al. *Biochemistry* 48 (2009) 9360). In more complicated lipid mixtures, we observe significant deviations from the binary PC/$PI(4,5)P_2$ titration curve. The hydrogen-bond donor lipid PE causes a large shift of the titration curve of the 4- and 5-phosphate of $PI(4,5)P_2$to lower pH values, indicating a stabilization of a more negatively charged form of $PI(4,5)P_2$. For mixtures of PC, PS, and $PI(4,5)P_2$, the titration curve appears to show a shift to higher pH values, indicating a decrease in negative charge for $PI(4,5)P_2$. This effect is pH dependent, with the strongest shift occurring in the

physiological pH range. In the presence of PI two opposing effects lead to a net charge that is similar to the charge found for $PI(4,5)P_2$ in the absence of PI. The enhanced negative charge in the membrane due to the presence of PI leads to an increased $PI(4,5)P_2$ protonation (reduced charge). This effect is opposed by PI/$PI(4,5)P_2$ hydrogen bond formation which results in increased deprotonation of the phosphomonoester groups. As a result, PI appears to have a minor effect on $PI(4,5)P_2$ ionization, however, fluorescence microscopy measurements of PC/PI/$PI(4,5)P_2$ GUVs show a pronounced effect on $PI(4,5)P_2$ morphology.

1120-Plat

Single Molecule Study of the Processive Ras/SOS Interaction

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Ras is a membrane-anchored small GTPase protein that plays an important role in regulating essential cellular functions such as proliferation, differentiation, and apoptosis. Its deregulation is a hallmark of many cancers and developmental defects. Son of Sevenless (SOS) is a guanine nucleotide exchange factor (GEF) enzyme that activates Ras by catalyzing the exchange of GDP to the GTP in Ras.

Previously, we have shown that in addition to the catalytic site, SOS has a catalytically inactive distal Ras-binding site, which allows SOS to localize and up-concentrate at Ras presenting membranes, dramatically increasing the Ras-GDP turnover rate. Together, the catalytic and allosteric sites form the catalytic core of SOS (SOScat). The existence of the extra binding site for Ras also raises the question of whether SOS is processive, capable of remaining surface bound via the distal binding site while catalyzing the nucleotide exchange of multiple Ras. In this study we employ various fluorescence-based methods such as Fluorescence Correlation Spectroscopy (FCS) and Total Internal Reflection Fluorescence (TIRF) microscopy on Ras functionalized supported lipid bilayers (SLBs) to demonstrate that SOScat is processive. Single molecule tracking of SOScat allows us to correlate the diffusion behavior between Ras and SOScat and further confirms the specific interaction.

By confining individual SOScat enzymes to micron-scale two-dimensional Ras-functionalized SLB ''reaction chambers'' we can simultaneously monitor enzymatic activity from hundreds of single SOScat, probing the variability in catalytic rate and processivity within the enzyme ensemble. Our data indicates that SOScat has a broad range of processivities ranging from a few up to a thousand turnovers.

1121-Plat

Partitioning of Cholesterol and Ganglioside GM1 in Phase Separated Lipid Bilayers Imaged by Secondary Ion Mass Spectrometry

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Secondary ion mass spectrometry has been used to image the distribution of cholesterol and ganglioside GM1 in model membranes composed of palmitoyl sphingomyelin (PSM) and dioleoylphosphatidylcholine (DOPC) best described as the canonical lipid-raft composition. Isotopic labeling or fluorination of each lipid bilayer component allowed the generation of molecule specific images using the NanoSIMS50L instrument at Stanford University. Simultaneous detection of six different ion species, including secondary electrons, were used to generate ratio images whose signal intensity values could be correlated to composition through the use of calibration curves from standard samples. Images of this system indicate the presence of three compositionally distinct phases corresponding to: 1) the interdomain region; 2) large domains ($d>3\mu m$); and, 3) small domains ($d=200nm\text{-}1\mu m$) localized within the large domains. Although semi-quantitative compositional analysis of these distinct phases suggests that both the small and large domains have similar cholesterol content, the large domains were also GM1 and DOPC-rich while the small domains were also PSM-rich. Since the interdomain region is primarily PSM-rich, this seems to suggest that the small domains do not correspond to kinetically trapped interdomain regions and that their more than doubled cholesterol content makes them instead a completely different phase. Furthermore, the average diameter ($d = 380nm \pm 230nm$) of these small domains suggests they might correspond to the formation of nanometer scale domains thus supporting the lipid raft hypothesis.

Symposium: Mitosis Studied with Biophysical Tools

1122-Symp

How Kinetochores Promote Accuracy in Mitosis: Tension, Phosphoregulation, and the Chinese 'Finger Trap' Effect

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An exquisite molecular machine, the mitotic spindle, organizes and separates chromosomes during cell division. To uncover how this machine operates we are reconstituting spindle functions and applying advanced biophysical tools for manipulating and tracking individual molecules. My talk will focus on kinetochores, the multi-protein organelles that link chromosomes to spindle microtubules, thereby driving chromosome movement. Kinetochores also perform vital regulatory activities that ensure the accuracy of mitosis. For example, a popular view is that tension selectively stabilizes proper kinetochore-microtubule attachments. Proper ('bi-oriented') attachments come under tension from opposing microtubules, and this tension is thought to cause their stabilization. Conversely, improper attachments lack tension, so they fail to become stabilized and detach quickly, giving another chance for proper attachments to form. We recently used laser trapping-based assays to show that tension stabilizes attachments between individual kinetochore particles purified from budding yeast and single microtubule tips. The tension in this case acts directly on the kinetochore-microtubule interface, causing it to adopt a more stable configuration in a manner similar to a Chinese 'finger trap' toy, or to the catch bonds that enhance cell-cell adhesion. Now we are investigating how this direct stabilization works in tandem with phosphoregulation. Aurora B kinase is known to promote mitotic accuracy through phosphorylation of kinetochore subcomplexes. Current efforts toward understanding how phosphorylation affects kinetochore function will be discussed.

1123-Symp

Visualizing the Interaction of Kinetochores Complexes with Microtubules

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Cryo-electron microscopy (Cryo-EM) has become a major tool in the structural characterization of large macromolecular assemblies, their architecture, interactions with different ligands, and the regulation of their function. I will present two different examples of how cryo-EM is being used in my lab to understand the molecular mechanisms of complex biological systems.

During division the eukaryotic cell needs to accurately segregate its genetic material between daughter cells. This process involves the interaction of the microtubule mitotic spindle with special regions on chromosomes called kinetochores. Errors, which result in misplaced chromosomes, can lead to cancer or death. We have visualized the interaction of microtubules with two kinetochore components, the yeast Dam1 and the human Ndc80 complexes, using cryo-electron microscopy and image reconstruction. Interestingly, both complexes oligomerize on the surface of the microtubule, a property that is essential for their capacity for harness the energy of microtubule depolymerization for chromosome movement.

1124-Symp

Systems Biochemistry and Structural Biology of Microtubule End Tracking

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The microtubule cytoskeleton performs essential mechano-chemical tasks in eukaryotic cells. It is crucial for the internal organisation of the cell, intracellular trafficking and for the separation of the genetic material during cell division. These complex processes require the coordinated activity of dynamic microtubules, molecular motors, their regulators, and other proteins linking microtubules to intracellular substructures. All these proteins form a dynamically interconnected microtubule cytoskeleton whose distinct biological function is intimately linked to its overall organisation and dynamic state. How this network operates as an integrative system and how its large-scale behaviour depends on the combinatorial action of its nano-scale biochemical constituents is a major open question. Biochemical reconstitutions of cytoskeletal subsystems mimicking in vivo behaviour, in combination with